REMARKS/ARGUMENTS

Claims 1-13 and 15-25 are pending in the present application and stand rejected on various grounds. No claim amendments have been made with the present response.

Claim Rejections - 35 U.S.C. §103

(1) Claims 1-13 and 15-24 have been rejected under 35 U.S.C. 102(a) as allegedly being unpatentable over Hart et al. (BIO/TECHNOLOGY Col. 12, November 1994) in view of the combined teachings of Wetzel et al. (EP 0155189) and Van Dien et al. (Appl. Environ Microbiol. 1997, 63(5):1689-95).

The Rejection

The rejection is essentially repeated from the Office Action mailed on March 17, 2008.

In particular, Hart et al. (especially pp. 1113-115) is cited for its teaching of a process for large-scale production of IGF-I from the periplasm of *E. coli* by culturing *E. coli* host cells having a plasmid comprising an inducible alkaline phosphatase promoter and nucleic acid encoding a human IGF-I linked to a *lamb* signal sequence for secretion into the periplasm.

Wetzel et al. (especially pp. 3-7 and claims 1-9) is cited for teaching a plasmid vector comprising an inducible promoter and nucleic acid encoding a T4 phage lysozyme.

Van Dien et al. (especially Results and Discussion and pp. 1689-1693) is cited for its alleged teaching of genes involved in polyphosphate metabolism in *E. coli* that were cloned behind different inducible promoters on separate plasmids.

According to the rejection, it "would have been obvious to one of ordinary skill in the art at the time the invention was made to place the nucleic acid encoding a T4 phage lysozyme taught by Wetzel et al. behind the arabinose inducible P_{BAD} promoter and/or place the nucleic acid encoding a human IGF-I linked to a *lamb* signal sequence for secretion into the periplasm taught by Hart et al. behind the IPTG inducible P_{tac} promoter," and to "further transform the *E. coli* cells of Hart et al. with the modified plasmid vectors of Wetzel et al. and/or the modified plasmid vector having the nucleic acid encoding a human IGF-I linked to a *lamb* signal sequence placed behind the IPTG inducible P_{tac} promoter." (Office Action, page 3, 3rd paragraph)

The Examiner asserts that one of ordinary skill would have been motivated to use lysozyme to rupture the polysaccharide membrane of the *E. coli* host cell after accumulation of human IGF-I in the periplasm, since this simplifies the purification of human IGF-I, and to wait until 50% or more of human IGF-I has accumulated before inducing T4 phage lysozyme expression in order to obtain a greater yield of human IGF-I. Motivation to place nucleic acid encoding the T4 lysozyme and nucleic acid encoding human IGF-I on the same vector is found since this approach "simplifies transformation in the *E. coli* host cell. (Office Action, pages 3 and 4)

The Examiner further notes that the art of recombinant heterologous protein expression in bacterial host cells "is well developed and widely used in biotechnology for obtaining a desired protein" (Office Action, page 4, 1st full paragraph), and cites Dennis et al. (WO 93/24633, published 12/09/1993) as allegedly showing that lysozyme was important in the purification and recovery of poly-β-hydroxybutyrate from *E. coli* host cells. (Office Action, page 4)

The rejection concludes with the statement that "the claimed invention was within the ordinary skill in the art to make and use at the time was made, and was as a whole clearly *prima facie* obvious." (Office Action, page 4, 3rd full paragraph)

Examiner's Answer to Applicants' Arguments

In addressing Applicants' arguments in response to an essentially identical rejection in the previous Office Action, the Examiner makes the following statements:

(1) one cannot show non-obviousness by attacking references individually;

(2) it is not necessary that the prior art suggest a cited combination of references to achieve the same advantage or result discovered by applicants;

(3) the Supreme Court in *KSR* reaffirmed the use of the *Graham* factors in obviousness determination; and

(4) "since an appropriate affidavit or declaration containing factual evidence that refutes, contradicts, and discredits the teachings and operability of the combination of the references has not been presented, then one of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success in obtaining a greater yield of human IGF-I by waiting until 50% or more of the human IGF-I has been accumulated before

inducing with arabinose to express T4 phage lysozyme." (Office Action, page 5, end of 1st paragraph)

Applicants' Response

The significant disagreement between the Examiner's and Applicants' position lies in the Examiner's assertion that at the time the invention was made one of ordinary skill in the art would have been motivated to wait until 50% or more of the human IGF-I has been accumulated before inducing lysozyme expression, and that the same skilled artisan would have had a reasonable expectation of success in obtaining a greater yield of IGF-I as a result of this measure. Throughout the long prosecution history of this application, in several Office Actions, the Examiner has failed to provide any evidence in support of this assertion.

Furthermore, even if one assumes arguendo that a motivation existed to improve the yield of human IGF-I production by delaying the production of phage lysozyme until 50% or more of human IGF-I, and that one of ordinary skill would have had a reasonable expectation to achieve this goal at the time the present invention was made, the finding of obviousness would still be unwarranted.

As explained in the specification, and as it is clearly reflected in the claims, the process disclosed and claimed in the present application requires the *coordinated expression* of two nucleic acid molecules, (1) nucleic acid encoding a desired heterologous polypeptide (e.g. IGF-I) and (2) nucleic acid encoding a phage lysozyme, in which *expression of the phage lysozyme is induced only after about 50% or more of the heterologous polypeptide has accumulated.*

There is nothing in the cited combination of references suggesting the coordinated expression of a nucleic acid encoding a heterologous protein and a nucleic acid encoding a phage lysozyme, where expression of the latter is induced only after about 50% or more of the heterologous polypeptide has accumulated. In addition, even if a motivation existed to delay the induction of phage lysozyme production until a late stage in the fermentation process, there was no reasonable expectation that this could have been accomplished without jeopardizing the success of the process as a whole.

As explained at page 9, lines 29-32 of the specification:

"...it would not be expected that induction at the end of a long fermentation process and after substantial product accumulation would produce enough of the phage lysozyme to be effective."

This statement is further explained and substantiated in the enclosed unsigned Declaration of Michael W. Laird, Ph.D. Applicants will file a signed Declaration shortly. Dr. Laird, a person skilled in the art of bacterial production of heterologous polypeptides, cautions that the timing for the induction of the phage lysozyme is a very delicate balance between guaranteeing that the cells have achieved an appropriate concentration of the recombinant product prior to lysozyme production and ensuring that there is enough lysozyme at harvest so that its effectiveness is not severely diminished. As Dr. Laird explains, it was known in the art at the time the present invention was made that high level production of recombinant proteins during fermentations is growth associated and occurs at its maximum rate during the exponential growth phase of the culture, and it was also known that bacterial cultures enter into stationary phase upon nutrient and energy depletion, and the machinery responsible for recombinant protein production is depleted in favor of maintaining the cellular metabolic activity. Before the invention disclosed and claimed in this application it was not known whether after accumulation of 50% or more of human IGF-I, when the nutrient and the energy pools are depleted to the point where cell survival is primary, the remaining cellular machinery would be able to produce sufficient amounts of the phage lysozyme, to be effective to perform its intended role. Therefore, as Dr. Laird explains, while waiting until accumulation of at least 50% of human IGF-I might have been desirable in order to increase IGF-I production, it was unpredictable whether this measure would not defeat the purpose of the process as a whole, which requires the production of the phage lysozyme in an amount sufficient to release retractile particles containing IGF-I from the cellular matrix or cell wall.

In view of the foregoing explanation, and the submitted declaratory evidence, the Examiner is respectfully requested to reconsider and withdraw the present rejection.

All claims pending in this application are believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited.

Please charge any additional fees, including any additional fees for extension of time, or credit overpayment to Deposit Account No. **50-4634** (Attorney's Docket No. **GNE-0128 A (123851-181805)**).

Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: August 17, 2009

By Electronic Signature: /GINGER R. DREGER/
Ginger R. Dreger
Reg. No. 33,055

GOODWIN PROCTER LLP
135 Commonwealth Drive
Menlo Park, California 94025
Telephone: (650) 752-3100
Facsimile: (650) 853-1038

LIBC/3656166.1